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Mammary arteriovenous differences of glucose, insulin, prolactin and IGF-I in lactating sows under different protein intake levels

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Abstract

Mammary uptake of nutrients is dependent on their availability in the circulation but the role of hormones in that process is not known. Arteriovenous differences (AVD) of glucose and key hormones across the mammary glands were therefore determined in sows fed varying levels of protein. Sixteen lactating sows (four/dietary treatment) were fed a 7.8, 13.0, 18.2 or 23.5% crude protein (CP) isocaloric diet throughout lactation and their litters were standardized to 11 pigs within 48 h of birth. The anterior main mammary vein and a carotid artery were cannulated on day 4 ± 1 of lactation and blood samples were collected every 30 min over 6 h on days 10, 14, 18 and 22 of lactation to measure glucose, insulin, IGF-I, and prolactin (PRL) concentrations. Amino acid data from these sows were previously published and used here to determine residual correlations. Dietary treatments had no effect on any of the insulin or PRL variables measured ($P > 0.1$) and, on day 18 only, IGF-I AVD was greater ($P = 0.05$) for sows on the 23.5% compared to the 18.2% diet. On days 18 and 22, sows fed the 13% CP diet had greater arterial, venous and AVD glucose concentrations than sows fed other diets ($P < 0.05$). Total arterial amino acid concentrations were correlated to arterial insulin ($P < 0.001$) and PRL ($P < 0.05$) concentrations, but not to those of IGF-I ($P > 0.1$). Mammary AVD for total ($P < 0.001$) and essential amino acids ($P < 0.05$) were correlated to arterial concentrations of insulin, but not to those of IGF-I ($P > 0.1$) or PRL ($P > 0.1$). Mammary AVD of both total ($P < 0.01$) and essential ($P < 0.05$) amino acids were also correlated to mammary PRL AVD. In conclusion, dietary protein level did not affect mammary AVD and circulating lactogenic hormone concentrations. Yet, amino acid utilization by the sow mammary gland seems to be regulated via both circulating insulin concentrations and PRL binding to and uptake by porcine mammary cells.

Keywords: Hormones; Lactation; Mammary; Protein intake; Sow

1. Introduction

The rate of milk protein and lactose synthesis depends on the availability of metabolic precursors such as free amino acids and glucose, respectively, in mammary tissue [1,2] and is regulated by lactogenic hormones. Prolactin (PRL) is known to be essential not only for the initiation but also for the maintenance of lactation in sows [3] and is involved in regulating the synthesis of major milk proteins including β-casein, whey acidic protein and α-lactalbumin [4]. The role of PRL in regulating the uptake of free amino acids and glucose by the sow mammary gland is poorly understood. In rodents, PRL stimulates the synthesis of lactose by increasing mammary glucose uptake [5]. Metabolic actions of

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other anabolic hormones, such as insulin and IGF-1, are highly associated with local mammary metabolism in the lactating sow [6]. More specifically, IGF-I stimulates mammary differentiation and lactogenesis [7], and overexpression of IGF-I in porcine lactating mammary tissue may regulate cellular transport systems [8]. Insulin has been shown to increase cell division *in vitro* in mammary tissue from lactating pigs [9]. In the rat mammary gland, insulin specifically increases the y^+ system-mediated uptake of arginine [10]. However, there is a scarcity of information regarding the role of insulin and IGF-I on nutrient uptake by the sow mammary gland.

Mammary uptake of amino acids in sows is dependent on their availability in arterial plasma [11,12], but the associated endocrinological processes have not been investigated. The objectives of the present study were therefore to (1) determine whether or not intake of dietary protein affects mammary arteriovenous differences (AVD) of key anabolic hormones (namely PRL, insulin and IGF-I) and glucose in lactating sows, and (2) determine whether or not mammary AVD of amino acids and glucose are related to circulating (arterial and venous) and AVD concentrations of PRL, insulin and IGF-I.

2. Materials and methods

This study was approved by the Michigan State University All-University Committee on Animal Use and Care.

2.1. Experimental design and diets

Sixteen Landrace × Yorkshire lactating sows (parity two or three) were allocated to dietary treatments according to a randomized block design. There were four blocks, where each block consisted of a time period. Within each block, four sows were provided *ad libitum* access to one of four diets containing, from a deficiency to an excess, 7.8, 13.0, 18.2 (normal), and 23.5% CP (as-fed basis). The 18.2% CP diet was formulated to meet the CP and lysine requirements of a sow nursing an average litter size of 10 pigs with an average daily gain of 200 g/pig [13]. To optimize similarity in amino acid concentrations relative to lysine across diets, corn and soybean meal were included in a fixed ratio of 1.05:1. Thus, the 23.5% CP diet was diluted with cornstarch and sucrose in a fixed ratio of 3:1 to obtain 18.2, 13, and 7.8% CP diets. Sucrose and tallow were used to improve palatability and decrease dustiness of diets resulting from the addition of cornstarch and solka flock. Diets were balanced to be isocaloric (14.3 MJ of ME/kg). Methionine, threonine, and valine were included so that all diets contained a minimum ratio of methionine, threonine and valine to lysine of 0.28, 0.68, and 0.88 [11]. Feed samples were finely ground using a Cyclotec 1093 Sample Mill (Foss Tecator, Hoeganaes, Sweden) and N was analyzed by the microKjeldahl method [14]. Ingredient and nutrient composition of diets were previously published [12] and are given in Table 1.

Table 1
Ingredient and nutrient composition of experimental diets (%, as-fed basis)

Ingredients	Dietary protein (%)			
	7.8	13.0	18.2	23.5
Corn	15.43	25.72	36.00	46.37
Soybean meal	14.75	24.58	34.41	44.32
Corn starch	42.53	28.39	14.25	0
Sucrose	14.18	9.46	4.75	0
Tallow	5.00	5.00	5.00	5.00
Solka flock	3.02	2.02	1.02	0
Dicalcium phosphate	3.14	2.64	2.14	1.63
Calcium carbonate	0.51	0.70	0.89	1.08
Salt	0.25	0.25	0.25	0.25
Trace and vitamin premix[a]	1.13	1.13	1.13	1.13
DL-Methionine	0.027	0.044	0.061	0.079
L-Threonine	0.011	0.019	0.025	0.033
L-Valine	0.038	0.064	0.090	0.115
Analyzed values				
Metabolic energy (MJ/kg)	14.3	14.3	14.3	14.3
Protein	8.2	13.2	18.2	23.0

[a] Provided the following amounts of trace minerals and vitamins: copper, 5 mg/kg; iodine, 0.075 mg/kg; iron, 50 mg/kg; manganese, 5 mg/kg; selenium, 0.15 mg/kg; and zinc, 50 mg/kg; retinyl acetate, 8.3 mg/kg; cholecalciferol, 0.0138 mg/kg; α-tocopherol, 44.1 mg/kg; menadione, 4.5 mg/kg; Vitamin B_{12}, 0.033 mg/kg; riboflavin, 4.5 mg/kg; D-pantothenic acid, 17.6 mg/kg; niacin, 26.4 mg/kg; thiamin, 1.1 mg/kg; pyridoxine, 1.0 mg/kg; choline, 385.0 mg/kg; folic acid, 1.65 mg/kg; and D-biotin, 0.22 mg/kg.

2.2. Animals

Litters were cross-fostered to 11 pigs per sow within 48 h of birth. Sows were housed individually in farrowing crates in a thermally controlled room (21 °C) and were provided *ad libitum* access to feed and water. Sows were provided with feed at 08:00 h, 12:00 h, 16:00 h and 20:00 h. Orts were collected daily and daily feed intake was recorded. Lights were turned on at 07:00 h and turned off at 20:00 h. All environmental conditions were consistent across blocks. The anterior main mammary vein and a carotid artery were cannulated on day 4 ± 1 of lactation as described by Trottier et al. [15]. At 24, 48, and 72 h after surgery, sows were administered an antibiotic (Naxcel, Pharmacia and Upjohn Co., Kalamazoo, MI) and an anti-inflammatory (Banamine, Schering-Plough Animal Health Corp., Kenilworth, NJ). Catheters were flushed once daily with heparinized saline (20 IU of heparin/ml).

2.3. Blood sampling

Carotid arterial and mammary venous blood samples were collected simultaneously every 30 min over 6 h from each sow on days 10, 14, 18, and 22 of lactation. Samples were kept on ice for a maximum of 30 min until centrifugation. Sows were fed 1 h before blood sampling (08:00 h feeding) and were provided *ad libitum* access to feed and water during the sampling period. Blood was centrifuged at $1500 \times g$ for 15 min at 4 °C, and plasma was removed and stored at −20 °C. For each sampling day, the 13 plasma samples obtained per sow were pooled for analysis.

2.4. Plasma analyses

Plasma concentrations of insulin were assayed in triplicate using a commercial porcine insulin RIA kit (Linco Cat. No. PI-12K, St. Louis, MO) and quantified using a 1290 Gamma Trac (Tm Analytic, Tampa, FL). The intra- and interassay coefficients of variation (CV) were 7.5 and 10.0%, respectively. Concentrations of IGF-I [16] and PRL [17] were determined with previously described RIAs. Extraction of IGF-I was performed using the formic acid–acetone method. The first antibody in the IGF-I assay and the radioinert prolactin were donated by A.F. Parlow (U.S. National Hormone and Pituitary Program, National Institute of Diabetes and Digestive and Kidney Diseases, Torrence, CA). The radioinert IGF-I was purchased from GROPEP (Adelaide, SA, Australia) and the first antibody to PRL was purchased from Research Products International (Mt. Prospect, IL). Parallelism of a plasma pool from lactating sows was demonstrated. Average recovery, calculated by addition of various doses of radioinert hormone to 50 μL of a pooled sample, were 97.1% for IGF-I and 103.5% for PRL. Sensitivities of the IGF-I and PRL assays were 62.5 pg/ml and 1.5 ng/ml, respectively. Six samples of a representative pool of plasma were carried in duplicates in all assays in order to calculate CV. The intraassay CV were calculated from the mean values of the pools within each assay: values were 3.1 and 8.1% for IGF-I and PRL, respectively. The interassay CV were calculated from the mean values of the pools obtained for each assay: values were 0.3 and 3.8% for IGF-I and PRL, respectively.

Plasma concentrations of glucose were determined in triplicate using a commercial kit (Sigma Procedure No. 315, St. Louis, MO) and read at 505 nm using a BU 7400 spectrophotometer (Beckman Instruments, Fullerton, CA).

2.5. Statistical analyses

Data (arterial concentrations, mammary venous concentrations and mammary AVD) were analyzed using the MIXED Procedure of SAS (SAS/STAT Version 9, SAS Institute, Cary, NC) and the first-autoregressive covariance structure as best fit. The model included the random effect of individual sow within dietary treatment and the fixed effects of block, dietary treatment, lactation day and all two-way interactions, with lactation day included in a repeated statement. All hormone and glucose variables were log transformed to satisfy the assumptions of homogeneous variance (Bartlett's test, $P > 0.2$) and normal distribution of residuals (Shapiro–Wilk's test, $P > 0.1$). Differences between all pair-wise mean comparisons within day of lactation were evaluated using Bonferroni adjustment for six contrasts. All least squares mean values are presented as back transformed least squares means with the standard error confidence intervals.

To explore relationships between mammary AVD of nutrients (i.e., glucose and amino acids) and insulin, PRL and IGF-I independently from random (sow) and fixed (diet and day of lactation) effects, correlations among model residuals were calculated. Amino acid concentrations in arterial and mammary venous plasma of the animals used in the present study were previously published [12] and were used to calculate the correlations.

3. Results

3.1. Insulin, PRL and IGF-I concentrations

Circulating concentrations and AVD for insulin, PRL and IGF-I as affected by dietary CP concentrations and day of lactation are shown in Figs. 1–3, respectively. Neither diets nor day of lactation affected any of the insulin variables measured ($P > 0.1$). Diets had no effect on either circulating or AVD concentrations of PRL ($P > 0.1$). Both arterial and venous concentrations of PRL decreased ($P < 0.001$) with increasing days of lactation (Fig. 2A and B). The large majority of mammary AVD values for PRL across days of lactation and diets did not differ from zero (Fig. 2C). Neither diets nor day of lactation affected any of the IGF-I variables measured ($P > 0.1$), except for IGF-I AVD where the 23.5% CP diet

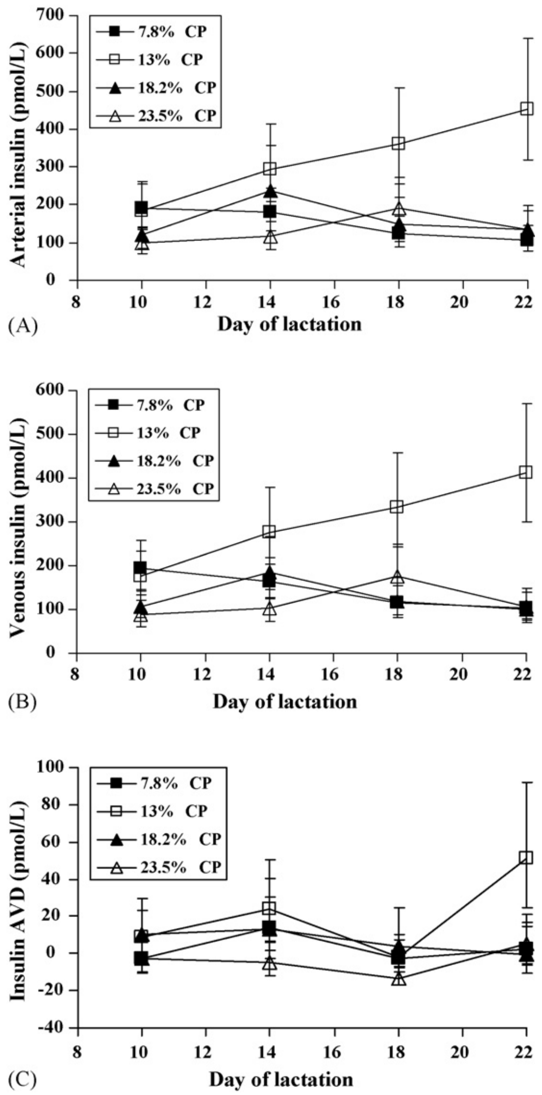

Fig. 1. Relationship between day of lactation and circulating insulin concentrations in sows fed varying concentrations of crude protein (CP). Data are back transformed from logarithm and are least squares means, with bars representing the confidence intervals. (A) Top panel: arterial concentrations; (B) middle panel: mammary venous concentrations; (C) bottom panel: mammary arteriovenous difference (AVD) concentrations. Arterial, venous and AVD insulin did not differ between days of lactation or diets (%CP).

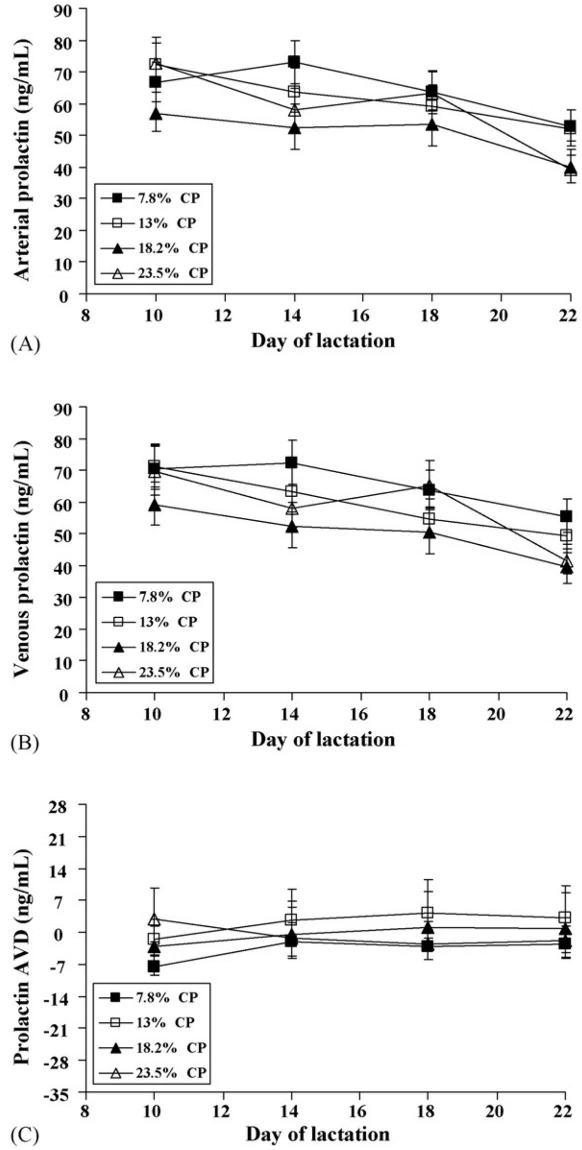

Fig. 2. Relationship between day of lactation and circulating prolactin (PRL) concentrations in sows fed varying concentrations of crude protein (CP). Data are back transformed from logarithm and are least squares means, with bars representing the confidence interval. (A) Top panel: arterial concentrations; (B) middle panel: mammary venous concentrations; (C) bottom panel: mammary arteriovenous difference (AVD) concentrations. Arterial and venous PRL decreased with day of lactation ($P<0.001$); AVD PRL did not differ between days of lactation. Arterial, venous and AVD PRL did not differ between diets (%CP).

differed from the 18.2% CP diet on day 18 ($P=0.05$). The large majority of mammary AVD values for IGF-I across days of lactation and diets did not differ from zero (Fig. 3C).

3.2. Glucose concentrations

Circulating concentrations and AVD for glucose are shown in Fig. 4. Dietary CP concentrations and day of lactation had no effect on circulating and AVD glucose concentrations except for sows fed the 13% CP diet. Sows fed the 13.0% CP diet had greater carotid arterial (Fig. 4A) and mammary venous (Fig. 4B) concentrations ($P<0.05$) as well as greater mammary glucose AVD ($P<0.05$; Fig. 4C) on days 18 and 22 of lactation.

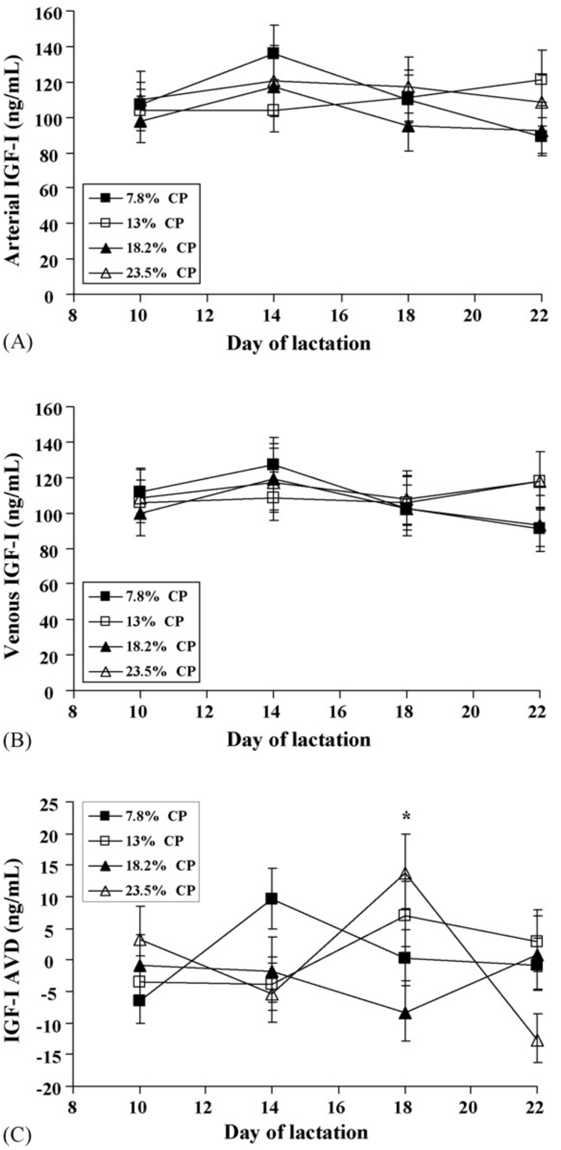

Fig. 3. Relationship between day of lactation and circulating IGF-I concentrations in sows fed varying concentrations of crude protein (CP). Data are back transformed from logarithm and are least squares means, with bars representing the confidence intervals. (A) Top panel: arterial concentrations; (B) middle panel: mammary venous concentrations; (C) bottom panel: mammary arteriovenous difference (AVD) concentrations. Arterial, venous and AVD IGF-I did not differ between days of lactation or diets (%CP). Diet × day interaction ($P < 0.05$) for IGF-I AVD, where 23.5% differs from 18.2% CP on day 18 ($^{*}P = 0.05$).

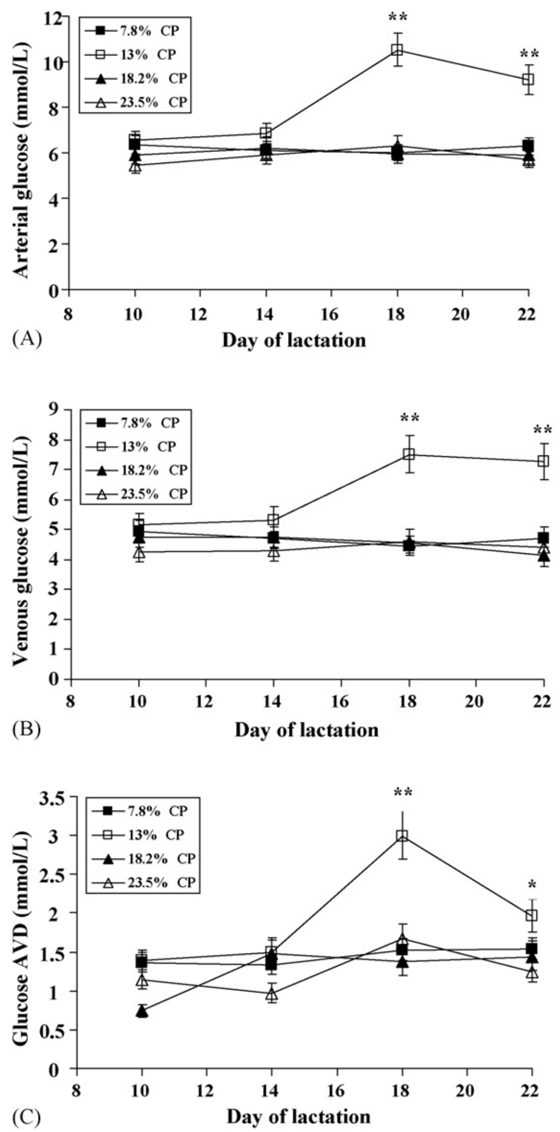

Fig. 4. Relationship between day of lactation and circulating glucose concentrations in sows fed varying concentrations crude protein (CP). Data are back transformed from logarithm and are least squares means with bars representing the confidence intervals (upper and lower values of the standard error of the mean). (A) Top panel: arterial glucose concentrations; (B) middle panel: mammary venous concentrations; (C) bottom panel: mammary glucose AVD (arteriovenous difference). Arterial, venous and AVD glucose did not differ between days of lactation or diets for diets 7.8, 18.2, and 23.5% ($P > 0.1$). Arterial and venous glucose differ ($^{**}P < 0.01$) between the 13% CP and the other diets on days 18 and 22 of lactation, and AVD glucose differs between the 13% CP and the other diets ($^{**}P < 0.01$) and the 13 and 23.5% CP diets ($^{*}P < 0.05$) on days 18 and 22 of lactation, respectively.

3.3. Correlations between blood concentrations or mammary AVD of hormones and mammary AVD of nutrients

Residual correlations between arterial and mammary venous concentrations for each individual variable measured were all significant ($P < 0.001$) and varied from 0.82 to 0.99 (data not shown). Correlations between arterial concentrations and AVD were significant for glucose ($r = 0.80$, $P < 0.001$) and insulin ($r = 0.61$, $P < 0.001$) but

Table 2
Partial correlation coefficients (r) between plasma mammary AVD for glucose and amino acids and plasma concentrations or mammary AVD for insulin, IGF-I and PRL[a]

	Plasma mammary AVD		
	Glucose	Total amino acids	Essential amino acids
Insulin			
Arterial	0.20	0.41**	0.42**
Venous	0.20	0.41**	0.42**
AVD	0.16	0.22	0.20
IGF-I			
Arterial	−0.11	−0.07	−0.07
Venous	−0.20	−0.04	−0.03
AVD	0.15	−0.06	−0.07
Prolactin			
Arterial	0.15	−0.04	0.10
Venous	0.15	−0.24	−0.07
AVD	−0.04	0.37**	0.30*

[a] Partial correlation coefficients between variables are adjusted for effects of dietary treatments and lactation periods, where $n = 50$.
* $P < 0.01$.
** $P < 0.05$.

not for IGF-I or PRL ($P > 0.1$). Arterial concentrations of glucose and insulin were also correlated ($r = 0.75$, $P < 0.001$). Total (essential plus non essential) arterial amino acid concentrations were correlated to arterial insulin ($r = 0.64$, $P < 0.001$) and PRL ($r = 0.34$, $P < 0.05$) concentrations, but not to those of IGF-I ($P > 0.1$). Residual correlations between AVD of nutrients and circulating concentrations of hormones are presented in Table 2. Mammary AVD of glucose were not correlated to any of the arterial, venous or AVD hormone concentrations. Mammary AVD of total amino acids ($P < 0.01$) and essential amino acids ($P < 0.01$) were correlated to arterial and mammary venous concentrations of insulin, but not to those of IGF-I ($P > 0.1$) or PRL ($P > 0.1$). Residual correlation data between total amino acids AVD and

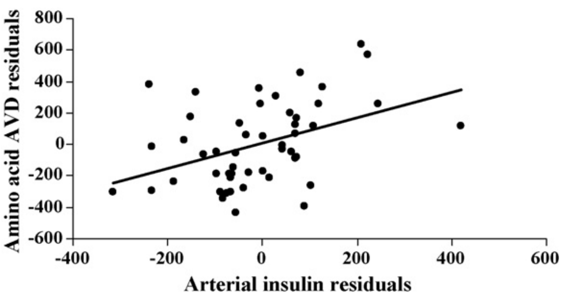

Fig. 5. Plot of residual correlations between total amino acids mammary AVD and arterial concentrations of insulin ($r = 0.41$, $P < 0.01$).

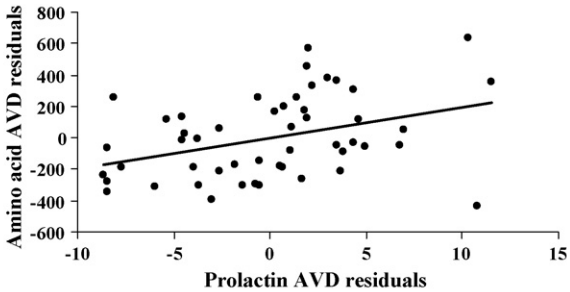

Fig. 6. Plot of residual correlations between mammary AVD for total amino acids and mammary AVD for PRL ($r = 0.37$, $P < 0.01$).

arterial concentrations of insulin are presented in Fig. 5. Mammary AVD of both total ($P < 0.01$) and essential ($P < 0.05$) amino acids were also correlated to mammary PRL AVD. Residual correlation data between mammary AVD for total amino acids and PRL are depicted in Fig. 6.

4. Discussion

Protein nutrition of the lactating sow affects mammary uptake of amino acids [2,11,12]. However, the effect of protein nutrition on lactogenic hormones and their potential role in regulating mammary nutrient transport, including amino acids and glucose, is unknown. The current study shows that overall, protein intake per se has little impact on circulating concentrations of the lactogenic hormones insulin, PRL and IGF-I, and of glucose. Albeit sows fed the 13% CP diet had greater circulating and AVD glucose concentrations at the end of lactation compared to other sows, it is unlikely that this response was related to protein intake in itself, based on the observation that there was no glucose response to other protein intake levels. Daily protein intake corresponded to 370, 750, 845, and 1070 g for sows fed the 7.8, 13, 18.2, and 23.5% CP diets, respectively. It is possible that circulating glucose concentrations and glucose transport were affected by the caloric ratio of protein to carbohydrate intake, as suggested previously [18].

In contrast to the response in insulin to protein intake observed herein, Kusina et al. [19] reported an increase in circulating insulin concentrations with a lactation diet providing 45 compared to 15 g of lysine per day. Similarly, a daily lysine intake of 37 compared to 22 g led to greater peripheral concentrations of insulin and heavier wet weight of mammary glands at weaning [20]. Pérez Laspiur et al. [21] also purported that dietary supplementation with crystalline L-arginine had beneficial effects on the catabolic state of lactating sows, in part, via alterations in insulin status. In this study, neither arterial nor AVD insulin concentrations were related to glucose AVD. Similarly, Holmes [22] found no change in mammary glucose AVD of lactating sows when concentrations of insulin in the blood were elevated during glucose infusion or following administration of insulin. Nonetheless, other studies have reported that sow milk yield increased following insulin injections [23] and that insulin concentrations were positively related to the major milk constituents in sows [24], indicating that insulin may have some control on the transport of nutrients in the mammary gland. In this study, arterial insulin concentrations were strongly correlated to amino acid AVD, indicating a role for insulin in the regulation of amino acid transport in mammary gland of lactating sows. For instance, supplementation of dietary amino acids coupled with a simultaneous infusion of insulin and glucose in lactating sows stimulated milk protein synthesis but not milk yield, whereas, infusion of insulin and glucose increased milk yield and milk lactose without altering milk protein secretion [25].

A lack of effect of protein intake on PRL concentrations was also demonstrated by Quesnel et al. [26] in a study where lactating sows were subjected to dietary protein restriction. The fact that PRL arterial concentrations were not correlated to mammary uptake of glucose corroborates findings in rats where mammary glucose AVD was not altered by PRL withdrawal for 24 h [27]. In contrast, PRL was shown to stimulate the uptake of deoxyglucose by mouse mammary gland explants [28]. The absence of correlation between circulating PRL concentrations and mammary uptake of amino acids or glucose in this study may be linked to the local production of PRL. Indeed, even though PRL is primarily secreted in the anterior pituitary, it is also secreted by mammary epithelial cells [29]. Therefore, *in situ* PRL synthesis may have confounded the mean mammary PRL AVD results in the present study since both negative (indicating mammary synthesis and secretion) and positive (indicating mammary uptake) AVD PRL values were obtained, resulting in mean AVD values not different from zero. Nonetheless, present data support the notion that PRL can be both taken up and synthesized by porcine mammary cells. Even though PRL is essential for the onset and maintenance of lactation in swine [3], increasing its concentration above a threshold does not have beneficial effects on milk yield [30]. In humans, it was also shown that only a small proportion of the PRL released at sucking is essential for the maintenance of milk secretion [31]. Plaut et al. [32] suggested that PRL binding was a better indicator of mammary metabolic activity than PRL concentrations in plasma. Consistent with this notion, while amino acid transport by the mammary gland was not correlated with plasma PRL concentrations, it was positively correlated with PRL uptake, and thus presumably with PRL binding. Thus, PRL and amino acid uptake or transport across the mammary gland may be coordinated and regulated events. In fact, the transcriptional regulation of the β-casein gene has been an excellent model to delineate the components of the PRL-signaling pathway [33].

The finding that protein intake did not affect circulating IGF-I concentrations corroborates previous results from Kusina et al. [19] and Clowes et al. [20]. On the other hand, Quesnel et al. [26] reported that dietary protein restriction throughout lactation reduced circulating IGF-I concentrations in sows of lighter body weight; as such, low body reserves may have led to an uncoupling between growth hormone and IGF-I secretions in order to facilitate mobilization of lean tissues. This, however, did not appear to be the case in the present trial. Results from the present study also demonstrate that dietary protein does not alter mammary uptake of IGF-I and that IGF-I does not appear to be taken up by lactating porcine mammary gland. On the other hand, the lack of apparent mammary uptake of IGF-I may, as for PRL, be due to local production in the mammary gland [7].

It can be concluded that dietary proteins fed at concentrations varying between 7.8 and 23.5% have no effect on mammary AVD and circulating lactogenic hormone concentrations in lactating sows. Prolactin AVD rather than circulating PRL concentrations were positively correlated to amino acid AVD, indicating that PRL binding to and uptake by porcine mammary cells is associated with amino acid transport across porcine mammary cells. Circulating insulin rather than insulin AVD was positively correlated to amino acid AVD, indicating that transport of amino acids across the mammary gland is also regulated, in part, by insulin. Thus, it seems that both insulin and PRL play some role in the regulation of amino acid transport by the porcine mammary gland *in vivo*, but the mechanism of transport regulation appears to differ. In fact, it has been well documented *in vitro* that insulin and PRL synergically stimulate β-casein synthesis [34]. Glucose AVD was poorly correlated with either circulating concentrations of insulin and PRL or insulin and PRL AVD, suggesting that glucose transport across the porcine mammary gland may be insensitive to insulin. Transport per se of neither glucose nor amino acids appears to be under IGF-I regulation. Finally, nutrient transport as measured by AVD may not be solely mediated through plasma concentrations and mammary AVD of the measured anabolic hormones and metabolites but other factors involving sensitivity of mammary tissue and receptors' binding affinity and capacity are undoubtedly involved.

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